<110> RIKEN

<120> Method for mutagenesis

<130> PH-1270US

<150> JP2000-237166
<151> 2000-08-04

<160> 9

<170> PatentIn Ver. 2.0

<210> 1
<211> 720
<212> DNA
<213> Aequorea victoria

<220>
<221> CDS
<222> (1)..(717)

<400> 1

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atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg   48
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1               5                   10                  15


gtc gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc   96
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Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
            20                  25                  30


gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc  144
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
            35                  40                  45


tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc  192
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
            50                  55                  60


ctg acc tac ggc gtg cag tgc ttc agc cgc tac ccc gac cac atg aag  240
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
    65                  70                  75                  80


cag cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag  288
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95


cgc acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag  336
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
                100                 105                 110


gtg aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc  384
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
            115                 120                 125


atc gac ttc aag gag gac ggc aac atc ctg ggg cac aag ctg gag tac  432
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
            130                 135                 140
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aac tac aac agc cac aac gtc tat atc atg gcc gac aag cag aag aac   480
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145             150             155             160


ggc atc aag gtg aac ttc aag atc cgc cac aac atc gag gac ggc agc   528
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165             170             175


gtg cag ctc gcc gac cac tac cag cag aac acc ccc atc ggc gac ggc   576
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180             185             190


ccc gtg ctg ctg ccc gac aac cac tac ctg agc acc cag tcc gcc ctg   624
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
            195             200             205


agc aaa gac ccc aac gag aag cgc gat cac atg gtc ctg ctg gag ttc   672
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
        210             215             220


gtg acc gcc gcc ggg atc act ctc ggc atg gac gag ctg tac aag taa   720
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225             230             235
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<210> 2
<211> 239
<212> PRT
<213> Aequorea victoria

<400> 2

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu

1   5   10   15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly

                20   25   30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile

                35   40   45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr

     50   55   60

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys

65   70   75   80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu

                85   90   95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu

                100   105   110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly

                115   120   125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr

                130   135   140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn

145   150   155   160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
          180   185   190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
          195   200   205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
          210   215   220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225   230   235

<210> 3
<211> 717
<212> DNA
<213> Aequorea victoria

<220>
<221> CDS
<222> (1)..(714)

<400> 3
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Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
    1   5   10   15

gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag   96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu

ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt att tgc   144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35   40   45

act act gga aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc   192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
        50   55   60

tct tat ggt gtt caa tgc ttt tca aga tac cca gat cat atg aaa cag   240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
    65   70   75   80

cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa aga   288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85   90   95

act ata ttt ttc aaa gat gac ggg aac tac aag aca cgt gct gaa gtc   336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100   105   110

aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa ggt att   384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
            115   120   125

gat ttt aaa gaa gat gga aac att ctt gga cac aaa ttg gaa tac aac   432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
        130   135   140

tat aac tca cac aat gta tac atc atg gca gac aaa caa aag aat gga   480

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145   150   155   160

atc aaa gtt aac ttc aaa att aga cac aac att gaa gat gga agc gtt 528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
              165   170   175

caa cta gca gac cat tat caa caa aat act cca att ggc gat ggc cct 576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
              180   185   190

gtc ctt tta cca gac aac cat tac ctg tcc aca caa tct gcc ctt tcg 624
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
          195   200   205

aaa gat ccc aac gaa aag aga gac cac atg gtc ctt ctt gag ttt gta 672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
          210   215   220

aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa taa   717
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225   230   235

<210> 4
<211> 238
<212> PRT
<213> Aequorea victoria

<400> 4
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu

                    20   25   30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys

                    35   40   45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe

          50   55   60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln

      65   70   75   80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg

                        85   90   95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val

                    100   105   110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile

                    115   120   125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn

          130   135   140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly

      145   150   155   160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val

                    165   170   175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
                180   185   190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
            195   200   205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
            210   215   220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225   230   235

<210> 5
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<220>
<223> Description of Artificial Sequence:Primer

<400> 5
gcactgcacg ccccaggtca gggtggt   27

<210> 6
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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Primer

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gggcggactg gtagctcagg tagtgg   26

<210> 7

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<221> Degenerate

<222>(9),(10) and (11)

<223> "n" is a, t, c or g

<220>

<223> Description of Artificial Sequence:Primer

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<210> 8

<211> 239

<212> PRT

<213> Aequorea victoria

<400> 8

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu

	1			5				10				15		

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
      20   25   30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
      35   40   45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
      50   55   60

Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65   70   75   80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
      85   90   95

Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
      100   105   110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
     115   120   125

Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130   135   140

Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145   150   155   160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
     165   170   175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180   185   190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
            195   200   205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
            210   215   220

Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225   230   235

<210> 9
<211> 239
<212> PRT
<213> Aequorea victoria

<400> 9
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
  1   5   10   15

Val Glu Leu Asp Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly
            20   25   30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
            35   40   45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
            50   55   60

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Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65              70              75              80


Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85              90              95


Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
                100             105             110


Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115             120             125


Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
        130             135             140


Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
145             150             155             160


Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165             170             175


Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
                180             185             190


Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
            195             200             205


Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210             215             220
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Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225   230   235